

Summary of *P. ramorum* trip to China:

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The USDA Forest Service has been building a productive working relationship with forestry officials in China over the past several years, spearheaded by our International Forestry (IF) staff with primary assistance from State and Private Forestry (S&PF) as well as Research and Development (R&D). The collaboration has been exceptionally positive in terms of bettering our understanding of insects and diseases (beech bark disease; longhorned beetle; emerald ash borer; gall wasp; hemlock woolly adelgid; white pine blister rust; etc.) of mutual concern with staff from Forest Health Protection (FHP) and Vegetation Management and Protection Research (VMPR) assisting IF with program development and implementation.

*P. ramorum*, a potentially devastating new pathogen, was first described in Europe on ornamental nursery stock (Werres et al. 2001). More recently, it has since been implicated in the death of thousands of oaks (*Quercus agrifolia* and *Q. kelloggii*) and tanoaks (*Lithocarpus densifloris*) in the western US (Rizzo et al. 2002). Although populations of *P. ramorum* in Europe and the US are largely distinct from one another, the organism is considered invasive in both locations with an operating hypothesis that it may be introduced. Asia is a center of diversity for many genera of plants and fungi, and more specifically the northern Yunnan Province of China has an abundance of rhododendrons and azaleas that have been collected for years by interested parties in the US and Europe. It is possible that *P. ramorum* may have originated somewhere in Asia and was unknowingly transported on commercial or privately collected ornamental plants. Therefore, there is a mutual interest to investigate forest *Phytophthora* spp. in China with the more immediate goal of doing an initial survey specifically for the pathogen *P. ramorum*.

There is a good deal of value in assisting the Chinese with their desire to determine if this serious pathogen or other *Phytophthora*'s exist in China. Ultimately, the hope was that this effort would help to evaluate the potential origins of *P. ramorum*, possibly leading to a better understanding of the role it plays in its natural environment. Such information could prove useful for future control efforts in the US and Europe. Natural biological control agents might be discovered or new host species might be identified. It could also help to avoid further introductions and identify significant trade routes that may act as possible pathways of further introduction to the US and Europe. However, maybe more realistically, this effort might lead to the identification of previously un-described *Phytophthora*'s, information about which might prove particularly valuable if introductions of these species were ever to occur elsewhere.

To meet the above objectives, two Forest Service scientists recently traveled to northern Yunnan Province in P.R. China to search for *Phytophthora*'s in natural forest ecosystems, more specifically to look for evidence of *P. ramorum*. International Forestry, using its network of contacts in China, arranged for field personnel in China to assist with site identification, visits, and sampling. The goal was also for the Forest Service scientists to

evaluate laboratory facilities, help assist Chinese colleagues develop and apply appropriate laboratory procedures (culturing, PCR, etc.) to identify *Phytophthora* spp., and to initiate further collaborations so that additional work could be conducted on *P. ramorum* and other *Phytophthora* spp. in China.

A two week trip was conducted from August 11-27, 2004. Gary Man, IF; Terry Shaw, VMPP; and Jerry Beatty FHP, S&PF helped coordinate the trip from the US and Dr. Zhao Wenxia coordinated most efforts within China. Dr. Zhao Wenxia is the Deputy Director of the Research Institute of Forest Ecology, Environment, and Protection (RIFEPP) and oversees the SFA Forest Pest Inspection & Identification Center. Ellen Goheen and Tom Kubisiak represented FHP S&PF and R&D, respectively.

The trip began in Beijing at the Chinese Academy of Forestry. Dr. Zhao coordinated a tour of the facility including the RIFEPP which includes two open research laboratories that belong to the State's Forestry Bureau and are affiliated to RIFEPP. We visited Dr. Zhao's laboratories which include the Forest Ecology and Environment Laboratory and Forest Protection Laboratory as well as a modern genomics laboratory in the Chinese Academy of Forestry.

In general, laboratories in the RIFEPP appeared to be scarcely equipped in terms of basic chemicals and equipment necessary to perform routine culturing and DNA-based diagnostics of *P. ramorum*. Most protocols, chemicals, and equipment were comparatively dated. Incubators, refrigerators, laminar flow hoods, microscopes, PCR machines, etc. exist but seem to be in short supply and of questionable condition/use. Interestingly, to make media for the field, cornmeal was cooked into a slurry and filtered through cheesecloth, the filtrate and agar were sterilized using a pressure cooker "bomb", and glass petri dishes were taken to the field. In stark contrast, other laboratory facilities, such as those in the Chinese Academy of Forestry, house a laboratory and training center with more advanced equipment for DNA, RNA, and protein analyses. Such equipment includes centrifuges, PCR thermal cyclers, real-time PCR machine, DNA sequencing gels, image analysis systems, a Proteomics workstation, liquid chromatography equipment, and equipment for microarray analyses. This laboratory certainly contains all the equipment necessary for performing most, if not all, of the current DNA-based diagnostics for *Phytophthora* spp. However, equipment appears to be in heavy use and high demand. The question we don't know is how much interaction there is between Dr. Zhao's laboratory and the genomics laboratory in the Chinese Academy of Forestry and how future use of the laboratory might be negotiated.

Our first field survey took us to Zhongdian in Yunnan Province (elevation ~11,000 ft). Travel plans to Zhongdian brought us through Kunming where we had a stop-over for several hours. While in Kunming we joined two contacts, Mr. Chay a Botanist and Mr. Wong a Forest Pest Specialist, both of whom would accompany us on to Zhongdian. We had lunch with the Director and ex-Director of the Yunnan Academy of Forestry, briefly discussed the plans of our trip, and departed later that evening for Zhongdian.

While in Zhongdian, we met Mr. Wong who was the Forest Pest Expert responsible for all of Diqing Prefecture. Mr. Wong arranged for us to visit three sample sites over a

three-day period: one was west to Bita Hai or the Bita Lake area; another was south to the Tiger Leaping Gorge area; and the third was north towards the border of Sichuan. After visiting a couple of the sites, one of the things that became apparent to Ellen and I was the remarkable similarity between the flora encountered in Yunnan and that found in the US. For the most part, many genera of overstory and understory plants were easy to identify, but the diversity and combinations of species in Yunnan was often very intimidating, even for Mr. Chay the botanist.

The first site at Bita Hai was at roughly 11,750 ft and in terms of hosts for *P. ramorum* it contained mostly *Quercus sinensis* and two different *Rhododendron* spp. One species of rhododendron appeared to be very healthy and displayed no visible symptoms of disease, the other species showed some symptomology and was sampled. Several other minor understory host species were present and some of these were symptomatic. At this site, symptomatic tissues sampled included shepards crooking, leaf tip dieback, leaf margin necrosis, and leaf spotting of *Rhododendron* spp.; shepards crooking and inner bark necrosis of *Quercus sinensis*; and leaf spotting of *Rosa* spp., *Fragaria* spp., and *Lonicera* spp.

The second site near Tiger Leaping Gorge was at roughly 6,000 ft and in terms of hosts for *P. ramorum* it contained mostly *Lithocarpus* spp. Several other minor understory host species were present. Symptoms at this site primarily included shepards crooking and leaf spotting of *Lithocarpus leucostachys* and leaf spotting of *Viburnum* spp.; *Rosa* spp.; and *Symphoricarpos* spp.

The third and final sampling effort was north of Zhongdian towards the Sichuan border, with most sampling sites varying from roughly 11,500 to 12,500 ft in elevation. These sites consisted mostly of various oak species with little to no rhododendron understory. Compared to the previous two sites, these sites were considerably more arid, possibly due to the aspect and elevation of the mountain ridges (?). Symptomatic tissues included primarily branch tip death on the shrub-like oak *Quercus monimotricha* (high-mountain oak) as well as leaf spotting, twig cankers, and branch tip dieback on *Quercus sinensis*.

After Zhongdian, we traveled back to Kunming where we had the opportunity to visit the Yunnan Academy of Forestry's Arboretum. The Arboretum was started in the 1950s and currently contains over 900 woody plant species (several provenances of each species) collected from throughout Yunnan and China. While in the arboretum, we noticed bleeding cankers on some of the *Quercus variabilis*. Further inspection suggested boring-insect damage, but three samples from the leading edge of a butt canker were sampled anyway. The rest of the day was spent touring the Key Laboratories for Rare and Endangered Forest Plants and Forest plant Cultivation and Utilization and discussing with the former Director of the Yunnan Academy of Forestry (Madam Li) more about the purpose of our visit, the situation with *P. ramorum* in the western US, and about various other research projects that we (Ellen and I) are involved with.

The next day was spent to the west of Kunming in the Xishan (West Mountain) Forest Park. It was a mixed forest type consisting primarily of *Keteleeria*, *Quercus*, and

*Lithocarpus* in the overstory and a very diverse understory consisting of some rhododendron. At this site symptomatic tissues sampled included stem canker of *Rhododendron decorum* and *Myrsine africana*; leaf tip necrosis on *Castanopsis delarayi* and *Berberis* spp.; and leaf spotting on *Quercus aliena* var. *acuteserrata*, *Lithocarpus dealbatus*, *Rhododendron decorum*, *Castanopsis delarayi*, *Machemia yunnanensis*, *Cyclobalanopsis* spp., and *Smilax* spp.

Prior to leaving Kunming we had breakfast with Dr. Zhao and the former Director of the Yunnan Academy of Forestry (Madam Li). The focus of the discussion at breakfast was the significance of our visit, the growing importance of *Phytophthora*'s in natural forest environments, and the need for further collaborations with scientists in both Yunnan Province and Beijing. We tried to emphasize the importance of China's participation in world discussions of forest *Phytophthora*'s, emphasizing how much China could contribute to that body of knowledge in future years. We discussed the reality of the situation, i.e. that *P. ramorum* might not be found on this visit, but that we hoped further efforts might be made to look for *P. ramorum*. We also tried to stress a need for characterizing *Phytophthora* spp. in general in natural forested ecosystems in China. Overall, Ellen and I both felt encouraged that Dr. Zhao and Madam Li both displayed a genuine interest in such a study provided that funds could be secured.

After each day of sampling, symptomatic plant tissues were recorded, photographed, and plated onto PARP medium. In total, 61 plates containing PARP medium and symptomatic plant tissues were taken back to the Chinese Academy of Forestry in Beijing for sub-culturing and DNA extraction. For some of the plates, especially those representing samples from the latter sites around Kunming, little growth of mycelia was noted. However, mycelia could be visibly seen growing from plant tissues on 31 of the 61 plates. Regardless of the presence of visible mycelia, all of the plant tissues contained within a single plate were pooled together and DNA was extracted using a standard CTAB-based extraction protocol. Since some symptomatic plant tissues collected in Kunming were taken back to Beijing, we also extracted DNA from an additional 30 samples. Unfortunately, there was only three days available in Beijing to sub-culture plates, do DNA extractions, and run a diagnostic assay. The DNA assay performed while in Beijing was not very informative, so DNA samples were brought back to the US for further analysis.

Although there is still more work to do in terms of further verification, 15 of the 92 samples assayed amplified very strong bands using the ITS6 and ITS7 primers described in Cooke et al. (2001). Unfortunately, none of the band profiles appear to match *P. ramorum*. However, interestingly many of the patterns are currently unknown, i.e., they don't match any of the ~25+ species of *Phytophthora* for which the SIFG has DNA. Dr. Frank Martin (USDA ARS) is putting together a collection of *Phytophthora*'s (isolates of all currently known *Phytophthora* spp. including isolates exhibiting intraspecific variation) so we will hopefully be able to get a better idea of what these samples may be in the near future. In the interim, our plans are to directly sequence the DNA in this region of ITS from the 15 samples in an attempt to identify them to the genus or species-level using sequence data.

To summarize, we feel the trip was successful for several reasons: the forests we saw in Yunnan were remarkably similar in genera yet profoundly different in terms of species diversity and composition relative to the west coast of the US; symptoms on the target species (*Quercus*, *Lithocarpus*, *Rhododendron* as well as minor SOD hosts such as *Lonicera*, *Smilacina*, etc) were abundant; we visited several locations with different host combinations (heavy to the *Quercus*...but unfortunately maybe a little light on rhododendrons and other ericaceous species); we were able to collect materials in the field, culture plant material at night, and process DNA in the lab in Beijing and bring an aliquot back to the US for further analysis; and most importantly we feel that there is a genuine interest and building enthusiasm for further cooperation on a similar project both in Yunnan and in Beijing. Possibilities (besides increased sampling of plants, soil, and water) include an interest in having someone from China come to the US to gain experience with the various diagnostic tests and *Phytophthora* identification. Dr. Zhao is currently writing a proposal to submit to the Chinese government for funding, and I am currently working on a proposal that could be used to pursue some funding on the US side.

More information will be coming regarding the 15 interesting samples.....